

Multidrug Resistance and Extended Spectrum Beta- Lactamase Producing *Escherichia coli* in Hostel

Restrooms

Abstract

Aim: The study was carried out to determine the prevalence of multidrug resistant and ESBL-producing *Escherichia coli* from inanimate surfaces in public and private restrooms within the University of Port Harcourt Nigeria.

Study Design: The employs experimental design and data collection

Place and Duration of Study: The study was carried out for a period of 9 months from December 2017 to August 2018 in the Medical Microbiology laboratory of the department of Microbiology, University of Port Harcourt Nigeria.

Methodology: A total of 105 samples were collected from floors, seats and door handles of the various toilets, the isolates were identified using standard microbiological methods. The positive cultures of *E. coli* were subjected to antimicrobial susceptibility testing using Gram negative disk by Kirby Bauer disk diffusion method. ESBL-producing *E. coli* were detected using several combinations of cephalosporin disks with clavulanic acid disks.

Results: Out of the bacteria identified from the swabbed area 33% (35isolates) were identified as *E. coli*. Antibiotic susceptibility testing showed high resistance of the isolates to Cefuroxime, Gentamicin, Cefixime, Augmentin, Ceftazidime, Ciprofloxacin, Ofloxacin. but were more susceptible to Nitrofurantoin. Ninety-five (95) % of isolated *E. coli* were resistant to at least resistant 3-5 antibiotics. The ESBL production of the isolated *E. coli* was noted from seats of both public and private toilets with 67% respectively than the floors with 20% and 33%. This study reveals ESBL producing *E. coli* can occur in large numbers on surfaces which users of toilets readily contact. **Conclusion:** Efforts should be made in monitoring the excessive use of antibiotics as these contributes to the resistance ability of the organism and also, daily cleaning and disinfection in conjunction with a regular hygiene service are recommended to prevent the spread multidrug resistant strains and ESBL producing *E. coli*.

Keywords: *Escherichia coli*, Extended Spectrum Beta lactamase, Inanimate surfaces, Multidrug Resistance, Toilets

1. INTRODUCTION

E. coli are responsible for urinary tract infection and other intestinal infections that can be expelled into the environment via fecal matter [1]. When implicated in infection they slow down effective treatment-based resistance to some broad-spectrum antibiotics. Antibiotic resistant strains of *E. coli* can spread through human population increasing diseases in the community and hospital [2]. ESBLs are Gram-negative bacteria that produce the enzyme beta-lactamase that has the ability to break down commonly used antibiotics, such as penicillin and cephalosporins and render them ineffective for treatment. ESBLs can be transmitted through unwashed hands by health care workers and individuals. They were initially associated with hospital- acquired outbreaks, currently ESBLs are now associated with fecal carriage in the community [3,4]. The increase of drug resistant organism has become a major challenge, because of this there is an increase of untreatable diseases in the world today, especially in developing countries. The fact that it takes years for new antibiotics to be produced

makes the situation alarming. Antibiotics inhibit the growth of susceptible bacteria while the once that are resistant tend to adapt to environmental conditions and can be the channel through which drug resistant strains and genes be transferred from environmental isolates to human [5,6]. Toilets are sanitation facilities at the user interface that permits the safe and convenient urination and defecation, toilets can be with or without flushing water. In private homes, the toilet, sink, bath, or shower may be in the same room. Another option is to have one room for body washing (bathroom) and a separate room for the toilet and hand washing sink (toilet room). Public toilets consist of one or more toilets which are available for use by the general public [7]. Aerosols toilets from door handles, tap head, sink, towels and floor are possible routes through which diseases can be transmitted [8]. This is possible because aerosols can persist for a minimum of 12 minutes after the toilet is flushed [9]. The toilets when flushed produce microdroplets which contain viable bacteria. These bacteria can rest on inanimate surfaces in the toilet or restroom as the case maybe. The bacteria can persist on inanimate surface for several days. Self-contamination can occur from touching such surfaces without washing hands after wards [6,10,11]. Contamination of the toilet environment has a key role to play in the transmission of infectious diseases.

2. MATERIALS AND METHODS

2.1 Description of Study Area and Sample Collection

A total of 105 samples were collected from door handles, seats, and floors of toilet facilities in Moses villa lodge Alakahia and Obi city lodge, Choba (private facilities). Samples were also collected from Nelson Mandela block A, C, D toilets and Ofrima girls' toilets (public facilities) in the University of Port Harcourt Rivers State Nigeria¹². The swabs were soaked in sterile tubes containing Brain Heart Infusion Broth (BHI) and transported immediately to the Medical Microbiology laboratory of University Port Harcourt and incubated for 24 h at 37°C.

2.1.1 Duration of Study: The study was carried out for a period of 9 months from December 2017 to August 2018 in the department of Microbiology, University of Port Harcourt Nigeria.

2.1.2 Identification of *Escherichia coli* Characterization and Identification of *Escherichia coli*

The bacteria isolates were grown on Eosin-Methylen Blue Agar (LAB M), they were sub cultured onto Nutrient Agar (LAB M). *E. coli* was characterized and identified based on their motility, microscopic morphology, colonial morphology and biochemical characterization as described in medical laboratory manual for tropic countries and taxonomic scheme of Bergey's Manual of Determinative Bacteriology [13,14] .

2.2 Antimicrobial Susceptibility Testing

All isolates were tested for the antimicrobial susceptibility through the Kirby Bauer diffusion according to the Clinical and Laboratory Standard guidelines¹⁵. The antimicrobial agents used in the susceptibility testing included Augmentin (30µg), Nitrofurantoin(30µg), Ofloxacin (5µg), Ceftazidime (30µg), Cefuroxime (30µg), aminoglycosides (Gentamicin (10µg) and a fluoroquinolone (Ciprofloxacin (5µg) (TOKU-E, USA). Inoculums were adjusted 0.5 McFarland turbidity standards on nutrient broth and swabbed across the entire surface of Muller Hinton agar plate. The plates were incubated within 15 minutes of the disk's application at 37°C for 18 to 24 hours. Isolates were considered as multidrug resistance (MDR), when it shows resistance to ≥ 3 antimicrobial agents [9, 15].

2.3 ESBL Screening and Detection

Detection of ESBL production was carried out on isolates that exhibited intermediate susceptibility/resistance to any one of the third generation cephalosporins ceftazidime / cefotaxime^{15, 16}. An inhibition zone of < 18mm for cefotaxime and <14mm for ceftazidime indicated that the isolated strain is an ESBL producer.

2.3.1 Double Disc Diffusion Testing

An overnight culture of identified isolates was inoculated onto Mueller-Hinton agar plates. Disks containing the standard 30 µg of ceftazidime, and ceftriaxone were placed 15 mm apart (edge to edge) and from an amoxicillin-clavulanic acid disk containing 10 µg was placed in the center of the plate. Following incubation for 16-20 hours at 35° C, zone of inhibition between a beta-lactam disk and that containing the beta-lactamase inhibitor indicated the presence of an ESBL.

2.3.2 Phenotypic detection of ESBLs by modified CLSI ESBL confirmatory Method

The modification of the CLSI ESBL confirmatory test was performed employing disks of Ceftazidime with or without Clavulanic acid, on which both boronic acid and EDTA were dispensed. Boronic acid (400µg) was dispensed onto commercially available antibiotic disks containing ceftazidime (30µg) with or without clavulanic acid (10µg)¹⁷. Additionally, 10µl of 0.1 M EDTA (containing 292µg of EDTA) was dispensed onto the same antibiotic disks on MHA plates inoculated with identified isolates. The agar plates were incubated at 37 °C for 18 hours. An augmentation of ≥ 5 mm in the growth inhibitory zone diameter of either ceftazidime-clavulanic in combination with boronic acid and EDTA, compared with the zone diameter of ceftazidime disk containing boronic acid and EDTA, was considered a positive result for ESBL production [17].

RESULTS AND DISCUSSION

Results obtained from the study showed that out of the 105 samples collected from both public and private toilet restrooms within the University of Port Harcourt 35 (33%) isolates were positive for *E. coli*. The isolates were identified by Standard Microbiological Technique and Biochemical tests. Sixty – seven parents (67%) of the

isolates showed growth of other organisms. The floors of both the public and private toilets were implicated with the highest carriage of the organism, having 61% for public toilets and 41% for private toilets respectively. The toilet seats had 39% public toilets and 29% for private toilets. *E. coli* isolates obtained from the door handles from the public toilets was 22% and 5.9% for private toilets. Antimicrobial susceptibility testing was carried out on 22 *E. coli* isolates from specific areas in public toilets. Results obtained showed that the highest level of resistance was in the isolates from toilet seats. They were 100% Cefuroxime, Gentamicin, Cefixime, Augmentin Ceftazidime, Ofloxacin, Ciprofloxacin and Nitrofurantoin was the least. The door handles were next in resistance and the floor isolates were least resistant to antibiotics. Confirmed *E. coli* isolates from specific areas in private toilets showed resistance pattern to various antibiotics disks, the highest level of resistance was observed in seat isolates followed by the door handles and the least resistance was found in the isolates from the toilet floors of the private toilet. The floor isolates differed only in the Nitrofurantoin.

Table 1 shows the multidrug resistant pattern of *E. coli* isolated from public and private toilets, all the isolates were resistant to CAZ and AMP. *E. coli* isolates from public toilets were more resistant to more than 3 antibiotics, 22 of the isolates were multidrug resistant. Nineteen of the isolates were resistant to 7 antibiotics, this confirms the high level of resistance observed in *E. coli* from the toilets. From the private toilets 13 of *E. coli* were resistant to more than 3 antibiotics as shown in table 1.

Table 1. Multidrug Resistant Pattern of *E. coli* in Public and Private Toilets Surfaces

S/N	Antibiotics	Nob. of MDR <i>E. coli</i> from Public toilet	Percentage of MDR <i>E. coli</i>
1	CRX-GEN-CXM-OFL-AUG-CPR-CAZ	19	86
2	CRX-GEN-CXM-AUG-CAZ	1	5
3	CRX-GEN-CXM-OFL-AUG-NIT-CPR-CAZ	2	9
S/N	ANTIBIOTICS	Nob. of MDR <i>E. coli</i> from Private toilet	Percentage of MDR <i>E. coli</i>
1	CRX-GEN-CXM-OFL-AUG-CPR-CAZ	11	85
2	CRX-GEN-CXM-AUG-CAZ	2	15

Key: MDR = Multidrug Resistant

Detection of Extended Spectrum Beta Lactamase Producing *E. coli* Isolates

ESBL detection was carried out on confirmed *E. coli* isolates, results obtained showed that 38% of the floor isolates and 25% of the seat isolates from the public toilets showed zone size of inhibition ≥ 22 mm around either Ceftazidime disc or combined disc of Ceftazidime and Clavulanic acid were considered as ESBL producer according to the Clinical Laboratory Standard Institute as shown in Table 1. ESBL was detected in all the sample sites of public toilets except for Nelson Mandela block C hostels as shown in Table 1. None of the door

handle isolates were ESBL producers. Results obtained also showed that 71% floor isolates and 60% seat isolate from private toilets were ESBL producers. The ESBL production of the *E. coli* was noted from seats of both public and private toilets with 67%. *E. coli* from floor and door handles showed 20% and 33% respectively (Table 1&2). For the private toilets results obtained showed that ESBL was detected all the isolates from Obicity lodge, it had the highest prevalence of ESBL producing *E. coli* as presented in Table 2.

Table 2: Phenotypic Detection of Extended Spectrum Beta Lactamase among *E. coli* from Public Toilets

Isolates code	Double Disc Synergy Diffusion Test (≥22mm)			Modified Phenotypic confirmatory test (≥5mm)		ESBL Production
	CTX (mm)	AMC (mm)	CAZ (mm)	CAZ (mm)	CAZ/CLAV (mm)	
NDTF01	6	R	12	20	16	-
NDTF02	8	R	24	14	20	+
NCTF14	2	R	22	30	22	-
NCTS14	6	R	20	26	20	-
NATS23	6	R	22	22	32	+
OGTF35	2	R	18	26	28	-
OGTF36	4	R	24	22	10	-
OGTS36	4	R	22	24	30	+

CTX; Ceftriaxone, AMC; Amoxiclav, CAZ; Ceftazidime, CAZ/CLAV; Ceftazidime/Clavulanic acid, R; Resistant, +; Positive, -; Negative

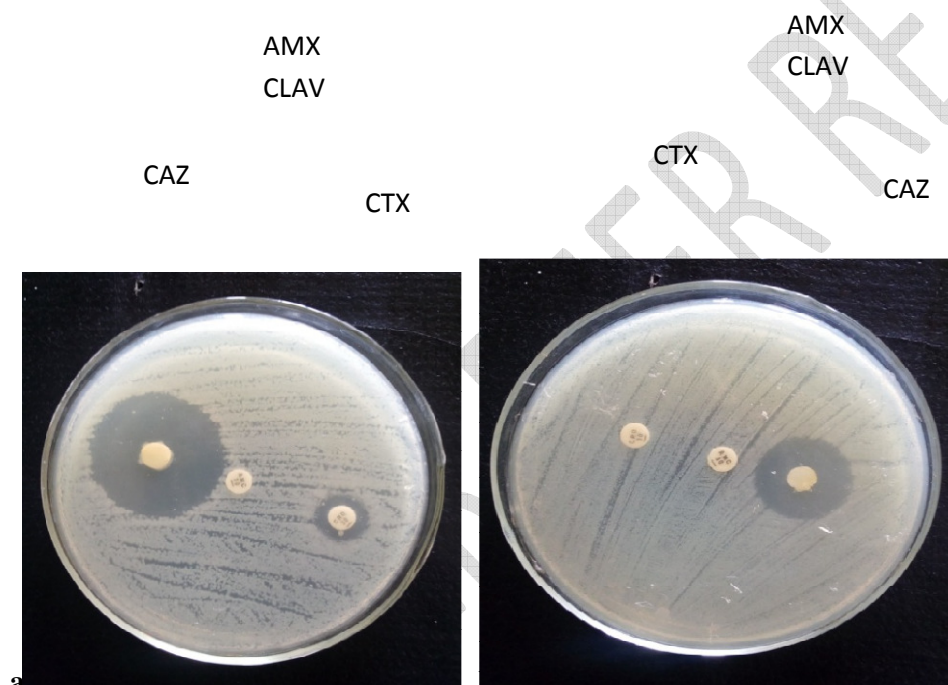
Table 3: Phenotypic detection of Extended Spectrum Beta Lactamase among *E. coli* from private toilets

Isolates code	Double Disc Synergy Diffusion Test (≥22mm)			Modified Phenotypic confirmatory test (≥5mm)		ESBL Production
	CTX (mm)	AMC (mm)	CAZ (mm)	CAZ (mm)	CAZ/CLAV (mm)	
OCLF20	6	R	22	14	22	+
OCLS20	2	R	24	26	38	+
OCLS40	6	R	24	22	38	+
MVLF12	2	R	20	24	26	-
MVLF14	2	R	20	18	12	-
MVLF15	4	R	26	24	38	+

DLAF21	4	R	26	28	24	-
DLAS21	2	R	22	16	12	-
DLAF26	6	R	26	12	8	-

CTX; Ceftriaxone, AMC; Amoxiclav, CAZ; Ceftazidime, CAZ/CLAV; Ceftazidime/Clavulanic acid, R; Resistant, +; Positive, -; Negative

Images of ESBL producing and non-ESBL producing *E. coli* from toilets are presented in plate 1. Isolate (a) NDTF02, indicative of ESBL production and isolate (b) DLAF21, negative for ESBL production. Isolate (c) NDTF02, confirmed ESBL producer and isolate (d) DLAF26, confirmed non-ESBL producer.



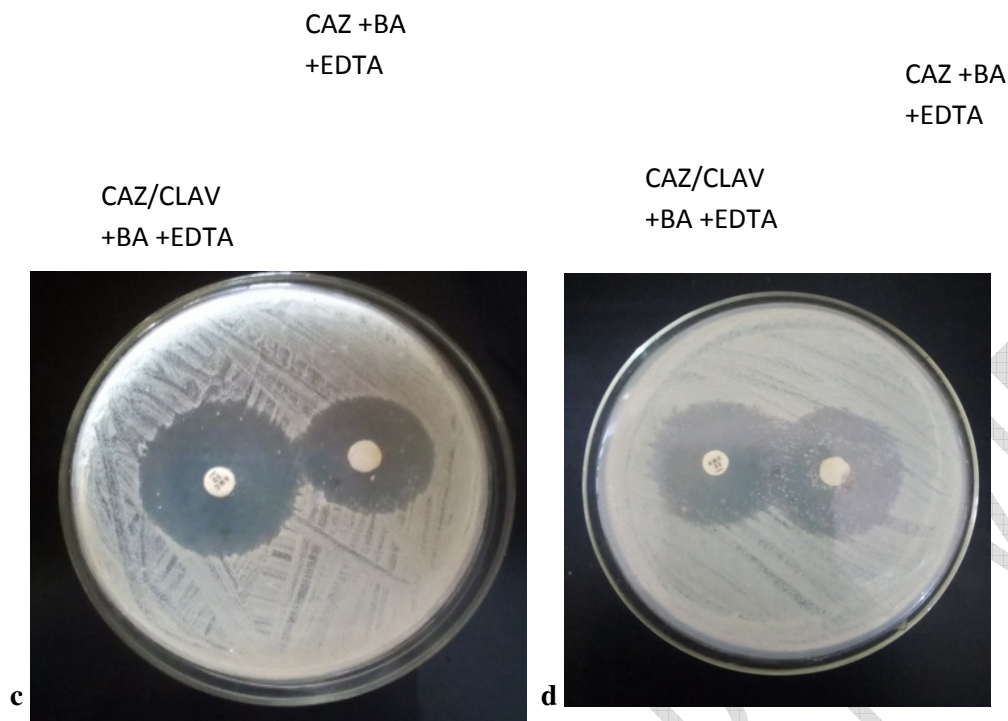


Plate 1: ESBL and non- ESBL producing *E. coli*

Infection caused by ESBL producing *E. coli* poses a great burden in respect to the control of infectious diseases due to multidrug resistance and the failure of the broad-spectrum antibiotics [18]. Resistant enterobacteria can persist on surfaces in restrooms. Some can be transferred from people that are fecal carrier of ESBL, the restroom environment contained with antibiotic residues exert selection pressure and contribute to the nature of resistant of bacteria associated with restroom surfaces [19]. The present study showed that variety of organisms are associated with restrooms surface, 33% were confirmed to be *E. coli* While 67% were other organisms. This is in agreement with the findings of Erb *et al* [20] where 24.3% Enterobacteriaceae were confirmed in 70 latrine samples and 16 *E. coli* identified. The percentage of *E. coli* in the present study is higher than those reported [20] which might be attributed to sample locations and the number of sampling surfaces within the restrooms. The floors of both the public and private toilets were implicated with the highest carriage of the toilets respectively, followed by the seats having 39% for public toilets and 29% for private toilets. *E. coli* isolates obtained from the door handles of public (22%) and private (5.9%) toilets were the least in occurrence compared to other areas. Other studies reported high occurrence of Enterobacteriaceae in the door handle and floors of the toilet. This can be attributed to the presence of faces or fecal matter on the floor of the restrooms, lack of running water or improper washing of hands contributes to the high occurrence of organism in door handles [20]. Gram negative microorganisms demands moist or damp sites for enhanced longevity. Recent reports suggest that *E.*

coli and *Klebsiella spp* may survive more than a year in moist surroundings, however *Serratia marcescens* can survive up to two months [21, 22]. The prevalence of ESBL from inanimate surface in the hospital environment is higher than those obtained from community sites. However, these results may vary depending on the studied location. Their study reported 94% multidrug resistance in *E. coli* and *Klebsiella pneumonia* [23]. In their study no resistance to carbapenems was detected, carbapenemase producers are found more among hospital isolates of *E. coli* and *Klebsiella* than community isolates [24]. A point of interest noted in the present study is that all the isolates were resistant to at least 3-5 antibiotics. The resistance was very high to Cefuroxime, Gentamicin, Cefixime, Augmentin, Ceftazidime, Ciprofloxacin, Ofloxacin but were more susceptible to Nitrofurantoin, which is comparable with the studies of Stoesser [25]. ESBL-producing *Escherichia coli* were detected using several combinations of cephalosporin disks with clavulanic acid disk. Of the four drugs tested, Ceftazidime with clavulanic acid was found to be the best ESBL detector for *E. coli*. Therefore, use of only one disk combination might fail to detect ESBL production resulting in under reporting of prevalence. Simultaneous use of four cephalosporin disks is recommended in screening for ESBL-producing organisms. ESBL production of the isolated *E. coli* was noted from seats of both public and private toilets with 67% for both (2 of 3 isolates) while the floors of the public and private toilets had 20% (1 of 5 isolates) and 33% (2 of 6 isolates) for ESBL production.

The finding in our study indicates the highest-risk areas for contamination with ESBL producing *E. coli* as seats of both public and private toilets and then the floors, suggesting that human feces are the main source for the recovered ESBL producing *E. coli*, as opposed to the organisms being free-living environmental strains [26]. *E. coli* is the most predominant bacteria isolated from stool, they are found in ESBL producing fecal carriage in stools of healthy people [27]. This explains the presence of ESBL producing Enterobacteriaceae in toilet sits. *E. coli* can adapt and colonize new human hosts, if acquired by toilet users. *E. coli* were recovered from door handles due to lack of washing of hands or improper washing of hands after defecating thus leading to a transfer of fecal matter from some persons to the door handle. This shows that toilets are important reservoirs for ESBL-producing bacteria, given their proven ability to survive on surfaces. Some studies have reported *E. coli* as the most prevalent Enterobacteriaceae in the stools of healthy people. Carbapenemase producers are more prevalent among hospital isolates of *Klebsiella* and *E.coli* than community isolated²⁴. In Fezcity a study was carried out to evaluate the efficacy of routine cleaning and disinfection practices of environmental surfaces in healthcare setting. Their study reported the bed rails (100%) were most contaminated with bacteria, this was followed by the bedsides (60%) and the least was reported in the door and room knobs (50%). *E. coli* was among the bacteria identified in the study [28]. Our study can be contrasted with prior related works, which includes three studies that investigated public restrooms [11, 29, 30]. Most studies carried out on restroom environment reported data mainly for *Staphylococcus* species and for general gut- and skin-associated taxa, providing limited data

specifically for *E. coli* [11,30,31]. For prevalence of contamination in relation to site within toilets, these two studies identified toilet-related sites as highest risk and identified contamination also of some non-toilet sites (hand dryer systems, inner door surfaces, taps, and soap dispensers) but provided no specific details for *E. coli*. This study identified high prevalence of multidrug resistant *E. coli* associated with restroom surfaces. Resistance of *E. coli* to several antibiotics decreases the effective treatment of infections associated with fecal *E. coli*.

CONCLUSION AND RECOMMENDATION

This study revealed the presence of ESBL producing *E. coli* on floors and seats of private and public toilet samples. Most of the *E. coli* were multidrug resistant and produced ESBL, ESBL- non-producing *E. coli* was higher than ESBL producing *E. coli*. This implies that beta lactamase alone is not the sole cause of resistance of *E. coli* from restroom surfaces to several antibiotics. It is recommended that further research should be done to type the strains of *E. coli* responsible for resistance in hospital and restroom surfaces. Daily cleaning and disinfection with regular hygiene service are recommended to prevent the spread of fecal *E. coli* present in restroom surfaces which were released via excreta.

SIGNIFICANCE STATEMENT

This study discovered the presence fecal *E. coli* that were resistant to 7 antibiotics, this is useful in the treatment of infectious diseases caused by fecal *E. coli*. This study will help the researchers to uncover the genes of fecal *E. coli* that are responsible for multidrug resistance, thus producing antibiotics that can target the genes responsible for the resistance. This will provide effective therapy for infections caused by ESBL producing *E. coli*.

REFERENCES

1. Foxman, B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Dis. Mon.* 2003, 49:53–70. DOI: [https://doi.org/10.1016/S0002-9343\(02\)01054-9](https://doi.org/10.1016/S0002-9343(02)01054-9)
2. Karlowsky, JA., Hoban, DJ., Decorby, MR., Laing, NM., and Zhanel, GG. Fluoroquinolone resistant urinary isolates of *Escherichia coli* from outpatients are frequently multidrug resistant: Results from the North American Urinary Tract Infection Collaborative Alliance-Quinolone Resistance study. *Antimicrob. Agents Chemo.*, 2006;50:2251–2254. DOI: [10.1128/AAC.00123-06](https://doi.org/10.1128/AAC.00123-06) MID:16723598 MCID: [PMC1479132](https://pubmed.ncbi.nlm.nih.gov/16723598/)
3. Schwaber, MJ. and Carmeli, Y. Mortality and delay in effective therapy associated with extended-spectrum beta-lactamase production in Enterobacteriaceae bacteremia: a systematic review and meta-analysis. *J. of Antimicrob. Chemo.*, 2007; 60:913–20. DOI: [10.1093/jac/dkm318](https://doi.org/10.1093/jac/dkm318).

4. Donskey, CJ. The role of the intestinal tract as a reservoir and source for transmission of nosocomial pathogens. *Clin. Infect. Dis.* 2004; 39:219–226. <https://www.ncbi.nlm.nih.gov/pubmed/15538978>
5. Wegener, H., Aarestrup, F., Gerner-Smidt, P., and Bager, F. Transfer of resistant bacteria from animals to man. *Acta. Vet. Scand.*, 1999; 92:51–58.
6. Kümmerer, K., and Henninger, A. Promoting resistance by the emission of antibiotics from hospitals and household into effluent. *Clin. Microb. Infec.*, 2003; 12:1203. DOI: <https://doi.org/10.1111/j.1469-0691.2003.00739.x>
7. Elizabeth, T., Luthi, C., Reymond, P., and Zurdrugg, D. Compendium of sanitation systems and technologies. Debendorf, Switzerland; Swiss Federal Institute of Aquatic Science and Technology, 2008; 9-151.
8. Clermont, O., Lavollay, M., Vimont, S., Deschamps, C., Forestier, C., Branger, C., Denamur, E., and Arlet, G. The CTX-M-15-producing *Escherichia coli* diffusing clone belongs to a highly virulent B2 phylogenetic subgroup. *J. Antimi. Chemo.*, 2008; 61:1024–1028. DOI: [10.1093/jac/dkn084](https://doi.org/10.1093/jac/dkn084)
9. Woerther P.L., Burdet, C., Chachaty, E., and Andremont, A. Trends in human fecal carriage of extended-spectrum beta-lactamases in the community: toward the globalization of CTX-M. *Clin. Microb. Revised.*, 2013; 26:744–58. DOI: [10.1128/CMR.00023-13](https://doi.org/10.1128/CMR.00023-13)
10. de Abreu, PM., Farias, PG., Paiva, GS., Almeida, .M., and Morais, PV. Persistence of microbial communities including *Pseudomonas aeruginosa* in a hospital environment: a potential health hazard. *BMC Microbiol.*, 2014; 8;14:118. DOI: [10.1186/1471-2180-14-118](https://doi.org/10.1186/1471-2180-14-118)
11. Flores, GE., Bates, ST., Knights, D., Lauber, CL., Stombaugh, J., Knight, R., and Fierer, N. Microbial biogeography of public restroom surfaces. *PLoS One.*, 2011; 6:e28132.
12. Norme ISO/DIS 14698-11999. Cleanrooms and associated controlled environments.
13. Cheesbrough, M. District laboratory practice in tropical countries. Cambridge University Press; 2004p. 357.
14. Bergey, DH1 and Holt, JG. Bergey's manual of determinative bacteriology. 9th ed. Baltimore: Williams & Wilkins. 1993.
15. Clinical and Laboratory Standards Institute. M100-S21. Performance standards for antimicrobial susceptibility testing; 21st informational supplement. Clinical and Laboratory Standards Institute, Wayne, PA. 2012.
16. Knothe, H., Shah, P., Krcmery, V., Antal, M., and Mitsuhashi, S. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. *Infection.*, 2005;11:315–317. DOI <https://doi.org/10.1007/BF01641355>.
17. Tsakris, A., Poulou, A., Pournaras, S., Volugari, E., Vrioni, G., Themeli-Digalaki, K., Petropoulou, D., and Sofianou, D. Use of boronic acid disk test to detect extended spectrum β -lactamases in

clinical isolates of KPC Carbapenemase possessing Enterobacteriaceae. J. Clini Microb., 2009;47:3420-3426. DOI: 10.1128/JCM.01314-09.

18. Sharma, M. Prevalence and antibiogram of extended spectrum Beta-lactamase (ESBL) producing Gram negative bacilli and further molecular characterization of ESBL producing *Escherichia coli* and *klebsiella spp.* J. and Diag. Pande. Microbm., 2013; 10:217- 377. Doi: 10.7860/JCDR/2013/6460.3462.
19. Ageroso, Y. and Sandvang, D. Class 1 integrons and tetracycline resistance genes in Alcaligenes, arthrobacter, and Pseudomonas spp. isolated from pigsties and manured soil. Appli. Enviro. Microbio., 2005; 71:7941-7947. DOI: 10.1128/AEM.71.12.7941- 7947.2005.
20. Erb, S., D'Mello-Guyett, L., Malebo, H.M., Njee, R.M., Matwewe, F., Ensink, J., Hinic, V., Widmer, A., and Frei, R. High prevalence of ESBL-Producing *E. coli* in private and shared latrines in an informal urban settlement in Dar es Salaam, Tanzania. Antimicrobial Resistance and Infection Control 2018; 7:3. DOI 10.1186/s13756-017-02.
21. Kramer, A., Schwebke, I. and Kampf, G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC. Infect. Dis., 2006; 6:130-136. <https://doi.org/10.1186/1471-2334-6-130>.
22. Silva-Sanchez, J., Garza-Ramos, JU., Reyna-Flores, F., Sanchez-Perez, A and Rojas-Moreno, T. Extended-spectrum β -lactamase-producing *Enterobacteriaceae* causing nosocomial infections in Mexico. A retrospective and multicenter study. Archived Med. Res., 2011; 42:156-162. DOI: [10.1016/j.arcmed.2011.02.004](https://doi.org/10.1016/j.arcmed.2011.02.004)
23. Tellevik, MG., Blomberg, B., Kommedal, O., Maselle, SY., Langeland, N., and Moyo. SJ. High Prevalence of Faecal Carriage of ESBL-Producing Enterobacteriaceae among Children in Dar es Salaam, Tanzania. PLOS ONE., 2016; 11(12): e0168024, <https://doi.org/10.1371/journal.pone.0168024>.
24. Cantón, R., Akóva, M., Carmeli, Y., Giske, C.G., Glupczynski, Y., Gniadkowski, M., Livermore, D.M., Miriagou, V., Naas, T., Rossolini, G.M., Samuelsen, O., Seifert, H., Woodford, N., and Nordmann, P. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. Clin. Microbiol. Infect., 2012; 18(5):413-31. <https://doi.org/10.1111/j.1469-0691.2012.03821.x>
25. Stoesser, N., Xayaheuang, S., Vongsouvath, M., Phommasone, K., Elliott, I., and Elias, CDO. Colonization with *Enterobacteriaceae* producing ESBLs in children attending pre-school, 2015; DOI: [10.1093/jac/dkv021](https://doi.org/10.1093/jac/dkv021).

26. Power, ML., Littlefield-Wyer, J., Gordon, DM., Veal, DA., and Slade, MB. Phenotypic and genotypic characterization of encapsulated *Escherichia coli* isolated from blooms in two Australian lakes. *Environ. Microbio.*, 2005; 7:631–640. DOI:[10.1111/j.1462-2920.2005.00729.x](https://doi.org/10.1111/j.1462-2920.2005.00729.x)
27. Luvsansharav, UO., Hirai, I., Niki, M., Nakata, A., Yoshinaga, A., Moriyama, T., and Yamamoto, Y. Prevalence of fecal carriage of extended-spectrum β -lactamase-producing Enterobacteriaceae among healthy adult people in Japan. *J. Infect. Chemother.*, 2011; 17(5):722-5.
28. Oumokhtar, D., Elouali Lalami, D., Benaicha, N., Arhoune, B., and Bono, W. Environmental Surfaces in Healthcare Setting: a great potential risk of pathogens transmission. *Biomed. Res.*, 2017; 28 (6): 0976 – 1683.
29. Mendes, M.F., and Lynch, DJ. A bacteriological survey of washrooms and toilets. *J. Hyg. (London)*, 1976; 76:183–190. <https://www.ncbi.nlm.nih.gov/pubmed/1063213>.
30. Mkrtychyan, HV., Russell, CA., Wang, N., and Cutler, RR.. Could public restrooms be an environment for bacterial resistomes? *PLoS* 2013; .ne8:e54223 .
<https://doi.org/10.1371/journal.pone.0054223>
31. Scott, E., Bloomfield, SF., and Barlow, CG. An investigation of microbial contamination in the home. *J. Hyg. (London)*, 1982 ; 89:279–293. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2134222/>.